<u>CLAIMS</u>

We Claim:

1.   A method for identifying one or more tissue-specific polynucleotides, said method comprising the steps of:

(a)   performing a genetic subtraction to identify a pool of polynucleotides from a tissue of interest;

(b)   performing a DNA microarray analysis to identify a first subset of said pool of polynucleotides of interest wherein each member polynucleotide of said first subset is at least two-fold over-expressed in said tissue of interest as compared to a control tissue; and

(c)   performing a quantitative polymerase chain reaction (PCR) analysis on polynucleotides within said first subset to identify a second subset of polynucleotides that are at least two-fold over-expressed as compared to said control tissue; wherein a polynucleotide is identified as tissue-specific if it is at least two-fold over-expressed by both microarray and quantitative PCR analyses.

2.   The method of claim 1 wherein said genetic subtraction is selected from the group consisting of differential display and cDNA subtraction.

3.   A method for identifying a subset of polynucleotides showing complementary tissue-specific expression profiles in a tissue of interest, said method comprising the steps of:

(a)   performing a first expression analysis selected from the group consisting of DNA microarray and quantitative PCR to identify a first polynucleotide that is at least two-fold over-expressed in a first tissue sample of interest obtained from a first patient but not over-expressed in a second tissue sample of interest as compared to a control tissue; and

(b)   performing a second expression analysis selected from the group consisting of DNA microarray and quantitative PCR to identify a second polynucleotide that is at least two-fold over-expressed in a second tissue sample of interest obtained from a second patient but not over-expressed in a first tissue sample of interest as compared to said control tissue;

wherein the first tissue sample and said second tissue sample are of the same tissue type, and wherein over-expression of said first polynucleotide in only said first tissue samples of interest and over-expression of said second polynucleotide in only said second tissue sample of interest indicates complementary tissue-specific expression of said first polynucleotide and said second polynucleotide.

4.   A method for determining the presence of a cancer cell in a patient, said method comprising the steps of:

(a)   obtaining a biological sample from said patient;

(b)   contacting said biological sample with a first oligonucleotide that hybridizes to a first polynucleotide said first polynucleotide selected from the group consisting of polynucleotides depicted in SEQ ID NO:73, SEQ ID NO:74 and SEQ ID NO:76;

(c)   contacting said biological sample with a second oligonucleotide that hybridizes to a second polynucleotide selected from the group consisting of SEQ ID NO: 1, 3, 5-7, 11, 13, 15, 17, 19-24, 30, 32, and 75;

(d)   detecting in said sample an amount of a polynucleotide that hybridizes to at least one of said oligonucleotides; and

(e)   comparing the amount of the polynucleotide that hybridizes to said oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

5.   A method for determining the presence or absence of a cancer in a patient, said method comprising the steps of:

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(a)   obtaining a biological sample from said patient;

(b)   contacting said biological sample with a first oligonucleotide that hybridizes to a first polynucleotide selected from the group consisting of polynucleotides depicted in SEQ ID NO:73, SEQ ID NO:74 and SEQ ID NO:76;

(c)   contacting said biological sample with a second oligonucleotide that hybridizes to a second polynucleotide as depicted in SEQ ID NO:75;

(d)   contacting said biological sample with a third oligonucleotide that hybridizes to a third polynucleotide selected from the group consisting of polynucleotides depicted in SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7;

(e)   contacting said biological sample with a fourth oligonucleotide that hybridizes to a fourth polynucleotide selected from the group consisting of polynucleotides depicted in SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:24;

(f)   detecting in said biological sample an amount of a polynucleotide that hybridizes to at least one of said oligonucleotides; and

(g)   comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

6.   A method for determining the presence or absence of a cancer in a patient, said method comprising the steps of:

(a)   obtaining a biological sample from said patient;

(b)   contacting said biological sample with an oligonucleotide that hybridizes to a tissue-specific polynucleotide;

(c)   detecting in the sample a level of a polynucleotide that hybridizes to the oligonucleotide; and

(d)   comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

7.   A method for monitoring the progression of a cancer in a patient, said method comprising the steps of:

(a)   obtaining a first biological sample from said patient;

(b)   contacting said biological sample with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein;

(c)   detecting in the sample an amount of said polynucleotide that hybridizes to said oligonucleotide;

(d)   repeating steps (b) and (c) using a second biological sample obtained from said patient at a subsequent point in time; and

(e)   comparing the amount of polynucleotide detected in step (d) with the amount detected in step (c) and therefrom monitoring the progression of the cancer in the patient.

8.   The method any one of claim 6 and claim 7 wherein said polynucleotide encodes a breast tumor protein selected from the group consisting of mammaglobin, lipophilin B, GABAπ (B899P), B726P, B511S, B533S, B305D and B311D.

9.   A method for detecting the presence of a cancer cell in a patient, said method comprising the steps of:

(a)   obtaining a biological sample from said patient;

(b)   contacting said biological sample with a first oligonucleotide that hybridizes to a first polynucleotide selected from the group consisting of mammaglobin and lipophilin B;

(c)   contacting said biological sample with a second oligonucleotide that hybridizes to a second polynucleotide sequence selected from the group consisting of GABAπ (B899P), B726P, B511S, B533S, B305D and B311D;

(d)   detecting in said biological sample an amount of a polynucleotide that hybridizes to at least one of the oligonucleotides; and

(e)   comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

10.   A method for determining the presence of a cancer cell in a patient, said method comprising the steps of:

(a)   obtaining a biological sample from said patient;

(b)   contacting said biological sample with a first oligonucleotide that hybridizes to a first polynucleotide selected from the group consisting of a polynucleotide depicted in SEQ ID NO:73 and SEQ ID NO:74 or complement thereof;

(c)   contacting said biological sample with a second oligonucleotide that hybridizes to a second polynucleotide depicted in SEQ ID NO:75 or complement thereof;

(d)   contacting said biological sample with a third oligonucleotide that hybridizes to a third polynucleotide selected from the group consisting of a polynucleotide depicted in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7 or complement thereof;

(e)   contacting said biological sample with a fourth oligonucleotide that hybridizes to a fourth polynucleotide selected from the group consisting of a polynucleotide depicted in SEQ ID NO:11 or complement thereof;

(f)   contacting said biological sample with a fifth oligonucleotide that hybridizes to a fifth polynucleotide selected from the group consisting of a polynucleotide depicted in SEQ ID NO:13, 15 and 17 or complement thereof;

(g)   contacting said biological sample with a sixth oligonucleotide that hybridizes to a sixth polynucleotide selected from the group consisting of a polynucleotide depicted in SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:24 or complement thereof;

(h)   contacting said biological sample with a seventh oligonucleotide that hybridizes to a seventh polynucleotide depicted in SEQ ID NO:30 or complement thereof;

(i)   contacting said biological sample with an eighth oligonucleotide that hybridizes to an eighth polynucleotide depicted in SEQ ID NO:32 or complement thereof;

(j)   contacting said biological sample with a ninth oligonucleotide that hybridizes to a polynucleotide depicted in SEQ ID NO:76 or complement thereof;

(k)   detecting in said biological sample a hybridized oligonucleotide of any one of steps (b) through (j) and comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value,

wherein the presence of a hybridized oligonucleotide in any one of steps (b) through (j) in excess of the pre-determined cut-off value indicates the presence of a cancer cell in the biological sample of said patient.

11.   A method for determining the presence of a cancer cell in a patient, said method comprising the steps of:

(a)   obtaining a biological sample from said patient;

(b)   contacting said biological sample with a first oligonucleotide and a second oligonucleotide;

i.   wherein said first oligonucleotide and said second oligonucleotide hybridize to a first polynucleotide and a second polynucleotide, respectively;

ii.   wherein said first polynucleotide and said second polynucleotide are selected from the group consisting of polynucleotides deptided in SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:76; and

iii.   wherein said first polynucleotide is unrelated in nucleotide sequence to said second polynucleotide;

(c)   detecting in said biological sample said hybridized first oligonucleotide and said hybridized second hybridized oligonucleotide; and

(d)   comparing the amount of said hybridized first oligonucleotide and said hybridized second hybridized oligonucleotide to a predetermined cut-off value; wherein an amount of said hybridized first oligonucleotide or said hybridized second oligonucleotide in excess of the pre-determined cut-off value indicates the presence of a cancer cell in the biological sample of said patient.

12.   A method for determining the presence or absence of a cancer cell in a patient, said method comprising the steps of:

(a)   obtaining a biological sample from said patient;

(b)   contacting said biological sample with a first oligonucleotide and a second oligonucleotide;

i.   wherein said first oligonucleotide and said second oligonucleotide hybridize to a first polynucleotide and a second polynucleotide, respectively;

ii.   wherein said first polynucleotide and said second polynucleotide are both tissue-specific polynucleotides of the cancer cell to be detected; and

iii.   wherein said first polynucleotide is unrelated in nucleotide sequence to said second polynucleotide;

(c)   detecting in said biological sample said first hybridized oligonucleotide and said second hybridized oligonucleotide; and

(d)   comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, wherein the presence of a hybridized first oligonucleotide or a hybridized second oligonucleotide in excess of the pre-determined cut-off value indicates the presence of a cancer cell in the biological sample of said patient.

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13.   A method for detecting the presence of a cancer cell in a patient, said method comprising the steps of:

(a)   obtaining a biological sample from said patient;

(b)   contacting said biological sample with a first oligonucleotide pair said first pair comprising a first oligonucleotide and a second oligonucleotide wherein said first oligonucleotide and said second oligonucleotide hybridize to a first polynucleotide and the complement thereof, respectively;

(c)   contacting said biological sample with a second oligonucleotide pair said second pair comprising a third oligonucleotide and a fourth oligonucleotide wherein said third and said fourth oligonucleotide hybridize to a second polynucleotide and the complement thereof, respectively, and wherein said first polynucleotide is unrelated in nucleotide sequence to said second polynucleotide;

(d)   amplifying said first polynucleotide and said second polynucleotide; and

(e)   detecting said amplified first polynucleotide and said amplified second polynucleotide;

wherein the presence of said amplified first polynucleotide or said amplified second polynucleotide indicates the presence of a cancer cell in said patient.

14.   The method of any one of claims 4-7 and 9-13 wherein said biological sample is selected from the group consisting of blood, serum, lymph node, bone marrow, sputum, urine and tumor biopsy sample.

15.   The method of claim 14 wherein said biological sample is selected from the group consisting of blood, a lymph node and bone marrow.

16.   The method of claim 15 wherein said lymph node is a sentinel lymph node.

17.   The method of any one of claims 4-7 and 9-13 wherein said cancer is selected from the group consisting of prostate cancer, breast cancer, colon cancer, ovarian cancer, lung cancer head & neck cancer, lymphoma, leukemia, melanoma, liver cancer, gastric cancer, kidney cancer, bladder cancer, pancreatic cancer and endometrial cancer.

18.   The method of any one of claims 12 and 13 wherein said first polynucleotide and said second polynucleotide are selected from the group consisting of mammaglobin, lipophilin B, GABAπ (B899P), B726P, B511S, B533S, B305D and B311D.

19.   The method of any one of claims 12 and 13 wherein said first polynucleotide and said second polynucleotide are selected from the group consisting of polynucleotide depicted in SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:76.

20.   The method of any one of claims 12 and 13 wherein said oligonucleotides are selected from the group consisting of oligonucleotides depicted in SEQ ID N0s:33-35 and 63-72.

21.   The method of any one of claims 12 and 13 wherein the step of detection of said first amplified polynucleotide and said second polynucleotide comprises a step selected from the group consisting of detecting a radiolabel and detecting a fluorophore.

22.   The method of any one of claims 4-7 and 9-13 wherein said step of detection comprises a step of fractionation.

23. The method of any one of claims 12 and 13 wherein said first and said oligonucleotides are intron spanning oligonucleotides.

24. The method of claim 23 wherein said intron spanning oligonucleotides are selected from the group consisting of oligonucleotides depicted in SEQ ID NO:36-62.

25. The method of claim 13 wherein detection of said amplified first or said second polynucleotide comprises contacting said amplified first or said second polynucleotide with a labeled oligonucleotide probe that hybridizes, under moderately stringent conditions, to said first or said second polynucleotide.

26. The method of claim 13 wherein said labeled oligonucleotide probe comprises a detectable moiety selected from the group consisting of a radiolabel and a fluorophore.

27. The method of any one of claims 4-7 and 9-13 further comprising a step of enriching said cancer cell from said biological sample prior to hybridizing said oligonucleotide primer(s).

28. The method of claim 27 wherein said step of enriching said cancer cell from said biological sample is achieved by a methodology selected from the group consisting of cell capture and cell depletion.

29. The method of claim 28 wherein cell capture is achieved by immunocapture, said immunocapture comprising the steps of:

    (a)   adsorbing an antibody to the surface of said cancer cells; and

    (b)   separating said antibody adsorbed cancer cells from the remainder of

said biological sample.

30.   The method of claim 29 wherein said antibody is directed to an antigen selected from the group consisting of CD2, CD3, CD4, CD5, CD8, CD10, CD11b, CD14, CD15, CD16, CD19, CD20, CD24, CD25, CD29, CD33, CD34, CD36, CD38, CD41, CD45, CD45RA, CD45RO, CD56, CD66B, CD66e, HLA-DR, IgE and TCRαβ.

31.   The method of claim 29 wherein said antibody is directed to a breast tumor antigen.

32.   The method of any one of claims 29-31 wherein said antibody is a monoclonal antibody.

33.   The method of claim 29 wherein said antibody is conjugated to magnetic beads.

34.   The method of claim 29 wherein said antibody is formulated in a tetrameric antibody complex.

35.   The method of claim 28 wherein cell depletion is achieved by a method comprising the steps of:
    (a)   cross-linking red cells and white cells, and
    (b)   fractionating said cross-linked red and white cells from the remainder of said biological sample.

36.   The method of claim 13 wherein said step of amplifying is achieved by a polynucleotide amplification methodology selected from the group consisting of reverse transcription polymerase chain reaction (RT-PCR), inverse PCR, RACE, ligase chain reaction (LCR), Qbeta Replicase, isothermal amplification, strand displacement

amplification (SDA), rolling chain reaction (RCR), cyclic probe reaction (CPR), transcription-based amplification systems (TAS), nucleic acid sequence based amplification (NASBA) and 3SR.

37. A composition for detecting a cancer cell in a biological sample of a patient, said composition comprising:

(a) a first oligonucleotide; and

(b) a second oligonucleotide;

wherein said first oligonucleotide and said second oligonucleotide hybridize to a first polynucleotide and to a second polynucleotide, respectively; wherein said first polynucleotide is unrelated in nucleotide sequence from said second polynucleotide; and wherein said first polynucleotide and said second polynucleotide are tissue-specific polynucleotides of the cancer cell to be detected.

38. The composition of claim 37 wherein said first polynucleotide and said second polynucleotide are complementary tissue-specific polynucleotides of the tissue-type of said cancer cell.

39. The composition of any one of claim 37 and claim 38 wherein said first polynucleotide and said second polynucleotide are selected from the group consisting of the polynucleotides depicted in SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:76.

40. The composition of any one of claim 37 and claim 38 wherein said oligonucleotides are selected from the group consisting of oligonucleotides as disclosed in SEQ ID NO: 33-72.

41.   A composition for detecting a cancer cell in a biological sample of a patient, said composition comprising:

(a)   a first oligonucleotide pair; and

(b)   a second oligonucleotide pair;

wherein said first oligonucleotide pair and said second oligonucleotide pair hybridize to a first polynucleotide (or complement thereof) and to a second polynucleotide (or complement thereof), respectively; wherein said first polynucleotide is unrelated in nucleotide sequence from said second polynucleotide; and wherein said first polynucleotide and said second polynucleotide are tissue-specific polynucleotides of the cancer cell to be detected.

42.   The composition of claim 41 wherein said first polynucleotide and said second polynucleotide are complementary tissue-specific polynucleotides of the tissue-type of said cancer cell.

43.   The composition of any one of claim 41 and claim 42 wherein said first polynucleotide and said second polynucleotide are selected from the group consisting of the polynucleotides depicted in SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:76.

44.   The composition of any one of claim 41 and claim 42 wherein said oligonucleotides are selected from the group consisting of oligonucleotides as disclosed in SEQ ID NO: 33-72.

45. A composition comprising an oligonucleotide primer or probe of between 15 and 100 nucleotides that comprises an oligonucleotide selected from the group consisting of oligonucleotides depicted in SEQ ID N0s:33-72.

46. The composition of claim 45 comprising an oligonucleotide primer or probe selected from the group consisting of oligonucleotides depicted in SEQ ID N0s:33-72.